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Sensory Effects of Transient Receptor Potential Channel Agonists on Whole Mouth Saliva Extensional Rheology

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Abstract

The extensional rheology (ER) of saliva is a property associated with its ability to coat surfaces and is important for the maintenance of a normal mouth feeling. Transient receptor potential (TRP) channels are expressed in the oral cavity and this study investigated how the sensory effects of TRP channel agonists modify the ER of saliva.

Healthy volunteers rinsed with solutions containing a TRP agonist. Unstimulated whole mouth saliva (WMS) was collected prior to rinsing and WMS was collected during the 1st and 2nd minutes after the mouth rinse. The Spinnbarkeit of the collected saliva was measured using a Neva Meter.

The nonivamide (TRPV1) mouth rinse increased WMS ER from 37.0 (\pm 6.3) mm to 49.3 (\pm 5.1) mm when compared to the vehicle control, which itself had no effect on WMS ER. However, this effect was short-lived and ER of WMS was not increased in the 2nd minute after the nonivamide mouth rinse. The menthol (TRPM8) mouth rinse resulted in an increase up to 57.8 (\pm 7.8) mm in WMS ER from the vehicle control and returned to control levels in the 2nd minute. The cinnamaldehyde (TRPA1) mouth rinse resulted in no change in WMS ER. It can be concluded that nonivamide and menthol mouth rinsing has a short term effect of increasing WMS ER, an effect not observed after cinnamaldehyde rinsing.

We hypothesise that the activation of some TRP channels in the oral cavity results in changes in the salivary protein composition that in turn alters WMS ER.

Practical Applications

Identifying compounds that modify the physical properties of saliva in a desirable way is important in developing treatments for conditions associated with changes in the physical properties of saliva such as xerostomia (also known as dry mouth). Furthermore, understanding the rheology of saliva contributes to the elucidation of food oral processing which is of importance to food manufacturers.

Introduction

Understanding the rheology of saliva is important in order to understand a multitude of oral processes including mastication, bolus formation and swallowing (Pedersen, Bardow, Jensen, & Nauntofte, 2002). Furthermore, the extensional rheology (ER) of saliva is responsible for its protective function on oral surfaces, texture perception, lubrication and mouth feel (Stokes & Davies, 2007). Therefore, identifying methods of modifying the rheology of saliva is important for developing treatments for conditions such as xerostomia (also known as dry mouth) and can contribute towards our understanding of food oral processing.

Whole mouth saliva (WMS) is a complex biofluid consisting of the secretions of the three major pairs of salivary glands, the submucosal minor glands, gingival crevicular fluid, serum transudate, microorganisms and mucosal epithelial cells (Beeley, 1993). It contains water, electrolytes and proteins which together are responsible for its rheology properties. Mucins are the salivary proteins known to be responsible for the extensional rheology of saliva (Stokes & Davies, 2007; van der Reijden, Veerman, & Amerongen, 1993) and are produced and secreted by the submandibular and sublingual glands, as well as most minor glands, which all contain mucous acinar cells (Proctor, 2016). Salivary mucins are heavily glycosylated proteins and give saliva the property of extensional rheology due to non-covalent bonding between glycosylated chains, with MUC7 having previously been demonstrated to be associated with Spinnbarkeit (Inoue et al., 2008). Moreover, salivary mucins play a major role in the formation and maintenance of the mucosal pellicle in the oral cavity (Gibbins, Yakubov, Proctor, Wilson, & Carpenter, 2014).

The extensional rheological of saliva can be assessed by measuring its Spinnbarkeit, which is the length at which a viscoelastic substance can be stretched out until its viscoelastic properties break down (Bhat et al., 2010). The Spinnbarkeit measurement of saliva is thought to be associated with its ability to coat oral surfaces and reduction in Spinnbarkeit is associated with xerostomia, Sjögrens syndrome and oral mucositis (Chaudhury, Shirlaw, Pramanik, Carpenter, & Proctor, 2015; Rossi et al., 2010).

TRP channels are a superfamily of non-selective cation channels that respond to a variety of somatosensory and endogenous stimuli. TRPV1, 3, 4, TRPA1 and TRPM8 (also called ThermoTRPs) are sensory TRP channels that are expressed in the mouth on mucosal and epithelial free afferent nerve endings of myelinated A δ and non-myelinated C fibres (Hand & Frank, 2014), oral epithelial cells (Ishida, Ugawa, Ueda, Murakami, & Shimada, 2002; Kido, Muroya, Yamaza, Terada, & Tanaka, 2003; Wang, Danjo, Kajiya, Okabe, & Kido, 2011), taste buds (Lyall et al., 2004; Smith, Treesukosol, Paedae, Contreras, & Spector, 2012) and keratinocytes (Ban et al., 2010). The thermoTRPs not only respond to temperature changes but also to chemical agonists and previous studies have shown that mouth rinsing with the TRPV1 agonists capsaicin, piperine and nonivamide leads to increases in WMS flow rate in human (Lawless, 1984; Nasrawi & Pangborn, 1990). Furthermore, it has been demonstrated that the salivary protein composition of rat saliva is modified after chronic exposure to a capsaicin rich diet (Katsukawa et al., 2002).

The aim of this study is to investigate whether TRP channel agonist containing mouth rinses alter the rheology of whole mouth saliva. The TRP channel agonists that were investigated were nonivamide (TRPV1 agonist), L-menthol (TRPM8 agonist) and cinnamaldehyde (TRPA1 agonist) (fig. 1).

[Figure 1 here]

Materials and Methods

Saliva Collection

Healthy volunteers (4 female, 2 male between the ages of 23 and 28) were recruited in accordance with the College's ethics policy. The volunteers were asked not to eat, drink or smoke during the one hour before the beginning of the experiment. The experiment was structured as follows (fig. 2): Unstimulated WMS was collected for 1 minute followed by 30 seconds of rinsing with 10 mL of a mouth rinse containing either 1 ppm nonivamide, 300 ppm cinnamaldehyde, 500 ppm L-menthol or a vehicle control. The vehicle control was propylene glycol (PG), which each of the three TRP agonists was solubilised in prior to dilution with water, diluted 3 in 100 with water which was the same as the least diluted TRP agonist mouth rinse. WMS was collected for the first and second minute after expectoration of the mouth rinse. Following a 15 minute break, the next mouth rinse was administered and the subsequent WMS collections carried out and the process repeated for the remaining two mouth rinses. The order of mouth rinses was randomised and the WMS samples were stored on ice immediately after collection.

[Figure 2 here]

Rheological Analysis

The extensional rheology of the collected WMS was measured using a Neva Meter (Ishikawa Iron Works, Japan) immediately after collection (Gohara et al., 2004). 100 μ L of the collected WMS was used for the measurement and each measurement made in triplicate. The extensional rheology was measured at the point when electrical conductivity of the saliva was broken after a constant stretching rate of 5 mm/s. The ER of the mouth rinse once it was expectorated was also measured but had no ER (data not shown).

Statistics

Data were tested for normality and analysed using one-way analysis of variance followed by the paired Student's t test between sample sets using GraphPad Prism 7 software with significance set for $P < 0.05$ (GraphPad Software Inc., La Jolla, CA, USA). Comparisons were made between the unstimulated WMS ER data and all other data (Un vs t=1/2), between WMS ER data collected after one or two minute/s of rinsing with PG and with WMS ER collected after one or two minute/s (respectively) of TRP agonist mouth rinsing (PG t=1 vs TRP agonist t=1/PG t=2 vs TRP agonist t=2), and between the WMS ER data one minute and two minutes after mouth rinsing of each mouth rinses (x t=1 vs x t=2).

Results

The mean (\pm SEM) ER of WMS during the first minute after both the nonivamide and menthol mouth rinses increased from the ER of saliva after rinsing with the PG control, up to 49.3 ± 5.1 mm ($P < 0.05$) and 57.8 ± 7.8 mm ($P = 0.06$, not statistically significant) respectively (fig. 3). This was followed by significant decreases in extensional rheology during the second minute, when compared to the first minute, after the nonivamide and menthol mouth rinses, reducing to 31.5 ± 2.8 mm ($P < 0.01$) and 43.8 ± 4.3 mm ($P < 0.05$) respectively. The mean ER of unstimulated saliva was observed to be 44.0 ± 5.9 mm and the only samples to show significant variation from that were the post-cinnamaldehyde and post-nonivamide mouth rinse samples taken during the second minute after mouth rinsing, both decreasing to 26.0 ± 2.2 mm and 31.5 ± 2.8 mm respectively ($P < 0.05$). Rinsing with cinnamaldehyde had no effect

on the ER of WMS secreted during the one minute after mouth rinsing and although a small decrease was seen in the ER of the saliva secreted in the second minute, when comparing to the secretions of the first minute, this was shown to be non-significant.

[Figure 3 here]

To ensure that the participants were experiencing a trigeminal stimulus; the participants were asked whether or not they perceived the mouth rinse to be burning, tingling, warming, cooling or numbing. All participants confirmed that the mouth rinses did elicit a trigeminal stimulus. Furthermore, panelists confirmed that they were no longer experiencing any trigeminal stimulation after the 15 min break.

Discussion

None of the mouth rinses significantly increase WMS ER above that of the unstimulated saliva. It is expected that unstimulated saliva would have the highest ER as the mucosal pellicle, which contains the mucins responsible for WMS ER, has not been disturbed by mouth rinsing. These mucins are contained in the expectoration and contribute to the WMS ER measurement. During mouth rinsing, the mucosal pellicle is disturbed and associated mucins are expectorated along with the mouth rinse and so are not contained in the sample collected 1 minute after mouth rinsing. Therefore, it is appropriate to compare the TRP agonist containing mouth rinses to the PG control mouth rinse to assess the effect of the TRP agonist on salivary secretion.

We hypothesise that the nonivamide and menthol containing mouth rinses are preferentially stimulating the salivary glands containing mucous acinar cells over the glands containing only serous acinar cells i.e. the submandibular and sublingual glands over the parotid glands. This phenomenon is also observed in the salivary secretory response to olfaction (Lee & Linden, 1992). During the one minute after the mouth rinse, storage granules containing mucins are secreted from the acinar cells resulting in an increase in WMS ER. However, over the second minute the glands are no longer being stimulated to secrete mucin containing storage granules resulting in the significant decrease in WMS ER compared to the first minute. It may even be the case that mucin stores have been depleted, as there is a significant decrease in WMS ER during the second minute after nonivamide mouth rinsing. Although it may appear that this data contradicts the results of Lawless *et al.*, who measure salivary flow for two minutes after nonivamide mouth rinsing and find that increasing concentration of nonivamide correlates with increased salivary secretion over two minutes, their results are not broken down into individual minutes so we cannot say during what time period the secretion is occurring and, furthermore, the concentration of nonivamide used is 5x that of this study (Lawless, 1984). It is clear that the cinnamaldehyde mouth rinse is not eliciting the same effect on the salivary glands as menthol or nonivamide and it may have inhibited mucin secretion as there is a significant decrease in WMS ER during the second minute after cinnamaldehyde mouth rinsing. This is despite the TRPA1 channel being expressed in the oral cavity and even co-expressed with TRPV1 on the same trigeminal afferent neurons (Story *et al.*, 2003). Therefore, the hypothesis cannot be extended to all TRP channel agonists and further investigation is required to elucidate the reason for this.

There are a number of studies that have used the same method of measuring Spinnbarkeit. Firstly, Chaudhury *et al.* observed the Spinnbarkeit of WMS from healthy individuals to be 28.5 ± 5.7 mm (Chaudhury *et al.*, 2015). The study also found the ER of WMS from xerostomic patients to be 9.2 ± 4.3 mm. Inter-individual variation in WMS ER is high, so we conclude that the ER values of the WMS from participants in this study falls within a healthy, non-xerostomic, range and are comparable to the

results of Chaudhury *et al.* Secondly, the mean ER of submandibular/sublingual saliva was found to be 38.1 ± 5.3 mm by Vijay *et al.* (Vijay, Inui, Dodds, Proctor, & Carpenter, 2015). Furthermore, Vijay *et al.* observed that a smelling stimulus increased the ER of submandibular/sublingual saliva compared to taste or mechanical stimuli and Zussman *et al.* observed that the viscoelasticity of submandibular/sublingual saliva was significantly greater than parotid saliva (Vijay *et al.*, 2015; Zussman, Yarin, & Nagler, 2007). The studies of Vijay *et al.* and Zussman *et al.* demonstrate that stimuli that primarily effect the mucin producing salivary glands are associated with increases in the extensional rheology of saliva. Finally, the studies of Inoue *et al.* and Gohara *et al.* observe unstimulated WMS Spinnbarkeit between a range of 1.9–4.9 mm, and a mean of 2.3 ± 0.4 respectively (Gohara *et al.*, 2004; Inoue *et al.*, 2008). However, it is possible that the measurement was not made immediately after collection of saliva which is important because in currently unpublished work, Vijay *et al.* have shown that the Spinnbarkeit of saliva reduces drastically after expectoration.

It can be concluded that nonivamide and menthol rinsing results in short term increases in WMS ER and we hypothesis that this is due to a secretion of saliva containing mucins. In the second minute after mouth rinsing with TRP agonists, the ER of the secreted WMS decreased from the first minute which may be indicative of a depletion of mucin stores after the initial short term stimulation.

Ethical Statements

The authors declare that they do not have any conflict of interest. This study was approved by the King's College London Ethics Committee (BDM/12/13-54).and written informed consent was obtained from all study participants.

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Figures

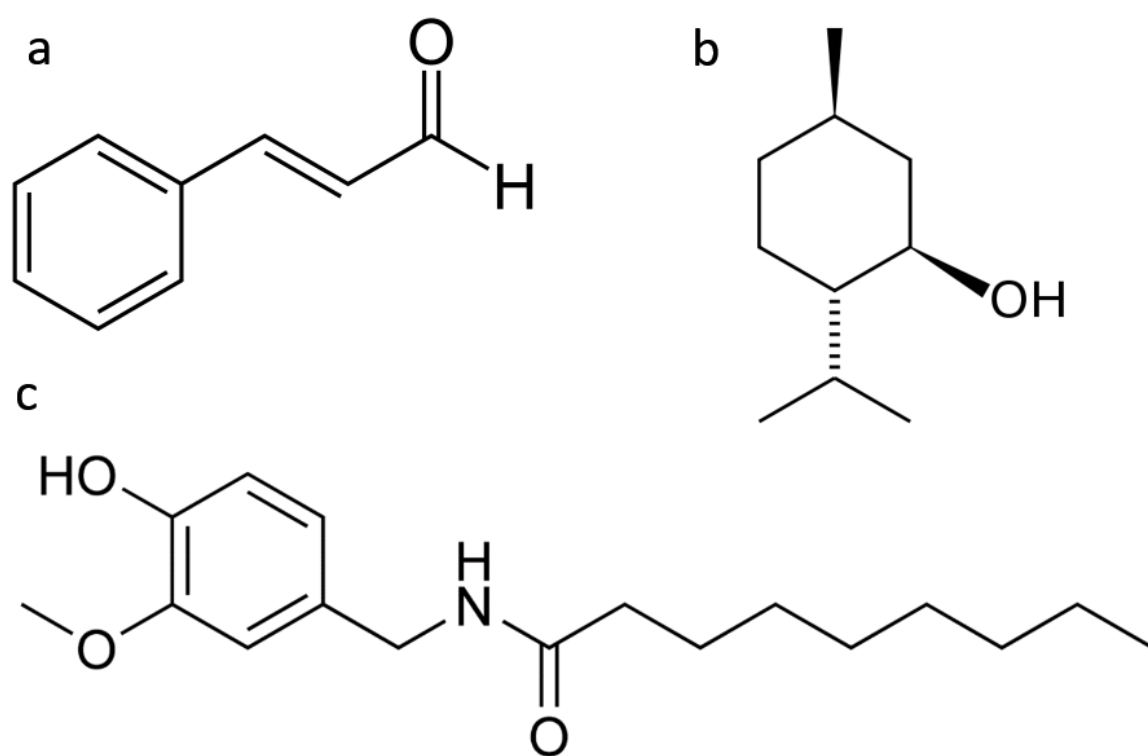


Figure 1. Chemical structures of **a)** cinnamaldehyde, **b)** L menthol and **c)** nonivamide.

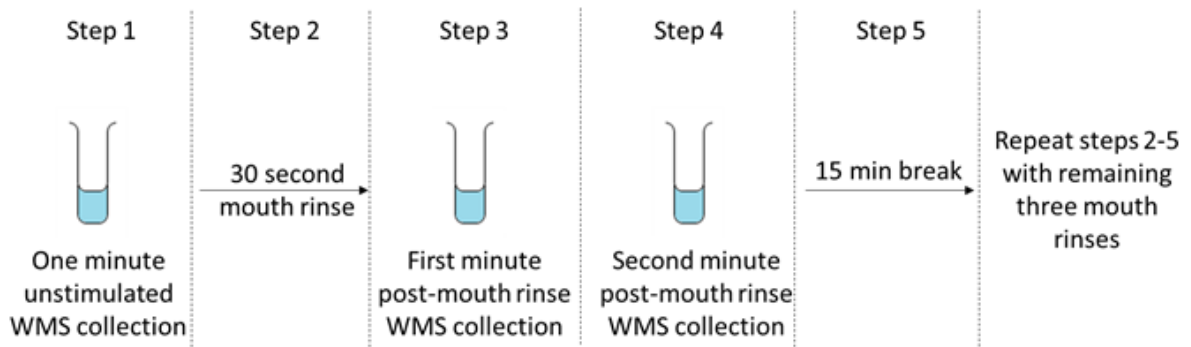


Figure 2. Schematic showing the method of saliva collection. 4 mouth rinses were administered in total containing either 1 ppm nonivamide, 300 ppm cinnamaldehyde, 500 ppm menthol or propylene glycol (diluted 3 in 100 times with water).

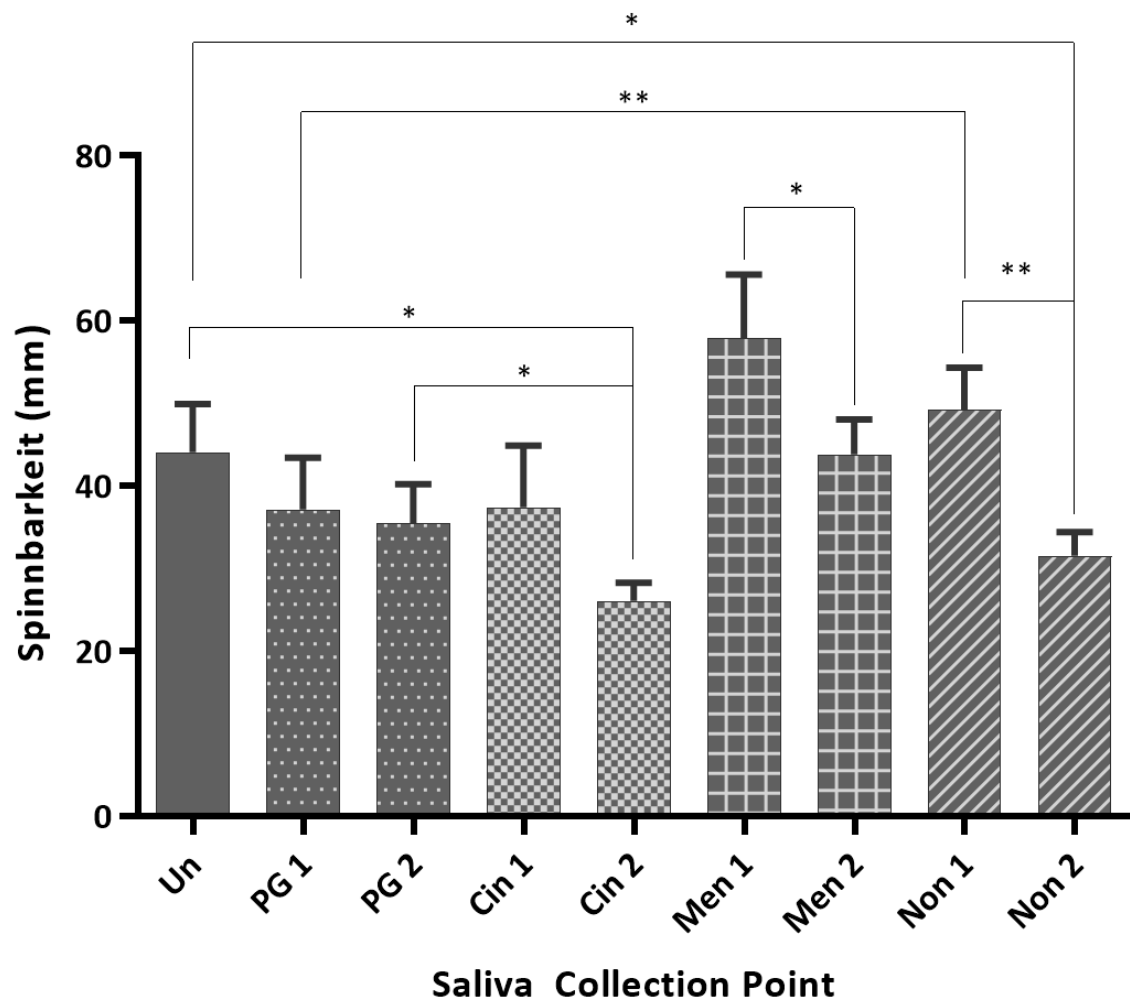


Figure 3. The mean (\pm SEM) spinnbarkeit of unstimulated whole mouth saliva (Un) or WMS collected during the minute after (1) or second minute after (2) mouth rinsing for 30 seconds with 10 ml of propylene glycol (PG), cinnamaldehyde (Cin), menthol (Men) or nonivamide (Non) (* P < 0.05, ** P < 0.01)).